

Minimal Residual Disease Assessment in CLL: Ready for Use in Clinical Routine?

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Abstract

The introduction of chemoimmunotherapy and more recently the implementation of novel agents into first-line and relapse treatment have substantially improved treatment outcomes in patients with chronic lymphocytic leukaemia (CLL). With longer progression-free survival and more frequently observed deep remissions there is an emerging need for sensitive methods quantitating residual disease after therapy. Over the last decade, assessment of minimal residual disease (MRD) has increasingly been implemented in CLL trials. The predictive value of MRD status on survival outcomes has repeatedly been proven in the context of chemoimmunotherapy and cellular therapies. Recent data suggests a similar correlation for Bcl-2 inhibitor-based therapy. While the relevance of MRD assessment as a surrogate endpoint in clinical trials is largely undisputed, its role in routine clinical practice has not yet been well defined.

This review outlines current methods of MRD detection in CLL and summarizes MRD data from relevant trials. The significance of MRD testing in clinical studies and in routine patient care is assessed and new MRD-guided treatment strategies are discussed.

Introduction

Treatment outcomes in chronic lymphocytic leukaemia (CLL) have improved considerably over the last decades with an increasing number of patients experiencing long lasting progression-free survival (PFS) after first-line therapy.¹ This undeniably positive development does, however, pose a significant challenge to clinical researchers when designing trials that compare new, potentially superior therapies against a highly effective standard treatment. A primary endpoint solely based on PFS or overall survival (OS) could excessively prolong the course of the clinical trial and delay approval of new treatments regimens.

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Minimal residual disease (MRD) in CLL is defined as the number of leukemic cells that can be detected in peripheral blood (PB) or bone marrow (BM) following treatment. Undetectable MRD (uMRD) is currently defined as the presence of less than 1 CLL cell in 10,000 leukocytes ($<10^{-4}$).²

When the first deep responses were achieved by combining fludarabine with cyclophosphamide (FC), detection of minimal residual disease slowly gained momentum in CLL.³ Over the last decades, the significance of MRD in CLL has steadily grown. Finally, several large randomized controlled trials (RCT) showed that MRD status after induction treatment is an independent predictor of survival and progression-free survival.^{4–13} Based on these data, in 2016 the European Medicines Agency (EMA) in contrast to the FDA allowed the use of uMRD as an intermediate endpoint in RCTs that were used for drug approval.¹⁴

While this regulatory decision has underscored the significance of MRD assessment in the context of clinical trials, it remains unclear whether routine MRD testing should also be implemented in clinical practice.^{15–17} In this review, we discuss current methods for MRD testing, MRD data from relevant trials, new MRD-guided treatment strategies and the role of MRD assessment in patient care outside of clinical trials.

Methods of MRD testing

The majority of CLL patients are not cured after frontline treatment but have some degree of residual disease.^{1,18} Over the last decades, residual disease status after treatment has been quantified by physical examination, blood counts, imaging studies and MRD status. MRD detection is performed using peripheral blood (PB) or bone marrow aspirate (BM). A compartment effect has been demonstrated with some substances being more effective in PB than in the BM. Hence, differences in the MRD levels of PB and BM can be observed, which are of relevant prediction for the duration of response.⁸

Table 1
Differences Between Multi-colour Flow Cytometry, RQ-PCR-, and HTS-Based Detection Methods

	Multi-colour flow cytometry	RQ-PCR	High throughput sequencing
Sensitivity (LOD)	4-color flow: confirmed 10^{-4} 6-color flow: reported 10^{-5}	confirmed 10^{-5}	reported 10^{-6}
Method	Surface antigen detection by different antibody combinations, e.g. CD5/CD19/CD20/CD43/CD79b/CD81	Detection of disease-specific <i>IGHV</i> using patient-specific primers	Detection of disease-specific IGH sequences after amplification of all IGH gene segments using consensus primers
Fresh material required?	yes, samples must be <48 h old	No, but DNA extraction preferably <48 h	
Standardized protocol?	yes	yes	ongoing
Advantages	Directly quantitative Widely available Results quickly available Highly standardized assay	High sensitivity No live leukocytes required	High sensitivity No live leukocytes required Multiple mutations can be detected in one test
Disadvantages	4-color flow: lower sensitivity Samples must be fresh	Not directly quantitative Requires baseline sample Time and labor intensive expensive	Not directly quantitative Requires baseline sample Less widely available expensive

LOD=lower limit of detection.

The established MRD detection level of $<10^{-4}$ in PB can currently be achieved by 3 different methods: multi-colour flow cytometry, real-time quantitative polymerase chain reaction (RQ-PCR) and high-throughput sequencing (HTS), each with their specific advantages (Table 1).

The European Research Initiative on CLL (ERIC) has established a harmonized multicolour flow cytometry (FCM) protocol that has been widely implemented and constantly optimized over the last years.^{19–22} The widespread use of this standardized assay in prospective CLL trials has led to highly comparable MRD results.

The antibody panel according to the ERIC protocol distinguishes B-cells from other leukocytes by using CD19 expression and differentiates normal B-cells from CLL cells based on their CD5, CD20, CD43, CD79b and CD81 expression.^{20,22} In contrast to PCR-based methods this assay does not need pre-treatment samples and can be adapted by most laboratories using cytometers with 6 or more colours. The panel can be extended beyond the 6 marker core panel by combination with additional markers, if necessary. Using this simple and reliable method, a detection limit of 10^{-5} can be achieved. Although studies correlating different MRD detection limits with clinical outcomes are sparse, it is conceivable that post-treatment MRD below a level of 10^{-5} or 10^{-6} might be associated with even longer survival when compared to standard uMRD as defined by the iwCLL guidelines ($<10^{-4}$).

A detection limit below 10^{-5} has also been reported for MRD assessment methods using RQ-PCR with patient-specific primers.^{23–27} In this method, the disease-specific *IGHV* is sequenced and allele-specific oligonucleotide (ASO) probes are designed in order to expand the malignant *IGHV* gene during PCR. As opposed to consensus PCR approaches, the assay using patient-specific primers and RQ-PCR methodology is quantitative. While this method also achieves a detection limit of 10^{-5} it is less widely used, as it is more time and labour intensive and costly. Moreover, the detection limit for each specific patient primer set has to be determined and *IGHV* sequencing before treatment start is necessary. Its main advantage compared to flow cytometry is that it can be performed on less material and non-fresh samples including frozen samples. In contrast to RQ-PCR approaches, digital droplet PCR (ddPCR) does not require preparation of a disease-specific reference standard curve. IGH-based MRD assessment methods using ddPCR are currently developed by numerous groups.

Another DNA-based method of MRD detection that does not require patient-specific primers is high-throughput IGH sequencing (IGH-HTS).²⁸ This method uses consensus primers for amplification of all *IGH* genes followed by HTS for quantification of the disease-specific *IGH* sequence that has been identified in pre-treatment blood samples.¹¹ With this approach, an MRD detection limit of 10^{-6} is reported to be achievable.²⁰

Multi-colour flow cytometry remains the most widely applied method for MRD assessment owing to its extensive availability, its degree of standardization, its reliable detection of currently applied MRD limits ($<10^{-4}$) and relatively low costs.¹⁹ In treatment strategies aiming at disease eradication (e.g. allogeneic stem cell transplantation [allo-HCT] or possibly in the future CAR T-cell therapy), there is a rationale for using more sensitive methods for MRD detection like HTS.

If future studies show that lower post-treatment MRD levels of $<10^{-6}$ translate into improved survival in CLL patients, HTS, alone or in combination with flow cytometry assays, could become the future gold standard of MRD assessment in CLL.

MRD assessment in clinical trials

MRD was assessed in a large number of pivotal CLL trials using diverse treatments and different MRD detection methods. In numerous of these trials, MRD correlated with survival (Table 2). Shown uMRD rates of randomized trials below in the text are based on Intention-to-treat (ITT) populations if not otherwise specified.

MRD with chemotherapy (CT) and chemoimmunotherapy (CIT)

Chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab (FCR) was the first treatment regimen besides allo-HCT showing remarkable rates of uMRD. In the German CLL Study Group (GCLLSG) CLL8 trial, MRD was analyzed in patients receiving FC or FCR treatment, with 35% (ITT-based: 12%) of FC-treated patients achieving uMRD ($<10^{-4}$) in PB vs 63% (ITT-based: 22%) after FCR chemoimmunotherapy.²⁹ uMRD after end of treatment (EOT) was associated with significantly longer PFS than intermediate ($\geq 10^{-4}$ to $<10^{-2}$) or high MRD ($\geq 10^{-2}$).⁴ Additionally, uMRD seemed to correlate better with PFS than clinical response assessment. Another

Table 2

Key Data Establishing the Association of Posttreatment MRD and Clinical Outcome.

Study	Study treatment	Method, threshold, sample source	uMRD rate based on		SPF	HR, p value
			MRD cohort ^a	ITT population ^b		
Böttcher et al 2012 ^a	FC or FCR	Flow, 10 ⁻⁴ , PB	FC: 51/147 (35%) FCR: 90/143 (63%)	51/409 (12%) 90/408 (22%)	68.7 (uMRD) vs 40.5 (intermediate) vs 15.4 months (high)	p < 0.001
Kovacs et al 2016 ⁸	FC or FCR or BR	Flow, 10 ⁻⁴ , PB	CR: 225 (82.6%) PR: 329 (48.9%)	NA	CR: median PFS: 61 (uMRD) vs 35 (MRD+ve) months. PR: median PFS: 54 (uMRD) vs 21 (MRD+ve) months.	CR: HR 1.99, p=.004 PR: not reported
Strati et al 2014 ¹³	FCR	Flow, 10 ⁻⁴ , BM	70/161 (43%)	70/237 (30%)	Not reported	uMRD vs MRD+ve: HR 0.1 (median NR), p=.03
Goede et al 2014, ⁷ Langerak et al 2018 ¹⁰	G-C1b or R-C1b	10 ⁻⁴ , FLC PB	G-C1b: 87/231 (38%) R-C1b: 8/243 (3%)	87/333 (26%) 8/330 (2%)	Median PFS: 56.4 (uMRD) vs 23.9 (intermediate) vs 13.9 months (high)	uMRD vs int. MRD: HR 2.65, p < .001; uMRD vs high MRD: HR 6.53, p < .001
Kwok et al 2016 ⁹	Any front-line	Flow, 10 ⁻⁴ , PB	55 (41%)	NA	10 y-PFS: 65% (uMRD) vs 10% (MRD+ve)	Not reported
Santacruz et al 2014 ¹²	Any treatment	Flow, 10 ⁻⁴ , PB	44 (17%)	NA	Median TFS: 76 (uMRD CR) vs 40 months (MRD+ve CR)	p < 0.001
Dreger et al 2010, ⁵⁶ Krämer et al 2017 ⁵⁷	Allo-HCT	Flow, 10 ⁻⁴ , BM	27/52 (52%)	27/100 (27%)	10-year relapse risk: 25% (uMRD) vs 80% (MRD+ve)	p < .0001
Logan et al 2013 ¹¹	Allo-HCT	HTS, 10 ⁻⁶ /quant. 10 ⁻⁵	15/31 (48%)	15/40 (38%)	50-month DFS: 93% (MRD < 10 ⁻⁶) vs 38% (MRD ≥ 10 ⁻⁶)	HR 7.9; p=.0002
Chanan-Khan et al 2016, ⁹³ Fraser et al 2018 ⁶	Ibrutinib + BR	Flow, 10 ⁻⁴ , PB/BM	76/211 (36%)	76/289 (26%)	2-year PFS: 91.5% (uMRD) vs 75.0% (MRD+ve)	Not reported
Wierda et al 2018 ⁴⁸	Venetoclax	Flow, 10 ⁻⁴ , PB	46/174 (26%)	46/285 (16%)	2-year PFS: 92.8% (uMRD) vs 84.3% (intermediate) vs 63.2% (high)	uMRD vs high: p < 0.001 Intermediate vs high: p=.005

uMRD: undetectable MRD (<10⁻⁴); MRD+ve: MRD positive (≥10⁻⁴); int. MRD: ≥10⁻⁴ and <10⁻²; high MRD: ≥10⁻²; Flow: multi-colour flow cytometry; TFS: Treatment-free survival; DFS: Disease-free survival.

^aPatients with available MRD testing.

^bAccording to iwCLL 2018 guidelines².

analysis also showed that patients with uMRD and a partial response (PR) due to residual splenomegaly had similar outcomes as patients with uMRD and a complete response (CR).⁸

The superiority of FCR in achieving uMRD was also shown compared to bendamustine/rituximab (BR) chemoimmunotherapy in the GCLLSG CLL10 trial. Patients treated with BR achieved uMRD less frequently than those who received FCR (ITT-based: 38% vs 49% in PB; p=0.041; MRD cohort: 63% vs 74%; p=0.029).³⁰

A predictive effect of PB uMRD was also shown in patients treated with chlorambucil and obinutuzumab (G-C1b) in the GCLLSG CLL11 trial.¹⁰ Patients achieving uMRD after EOT showed significantly prolonged PFS compared to those with positive MRD testing (not reached [NR] vs 19.4 months).⁷

On the basis of these 3 trials (CLL8, CLL10, CLL11), Dimier et al designed a predictive model, that for the first time quantitated the relationship between the effect of treatment on MRD and the effect of treatment on PFS.⁵ Consequently it allows prediction of treatment effect on PFS using treatment effect on PB MRD, which will prove useful in the design of MRD-based end points and the calculation of sample sizes in large clinical trials.³¹ As the prediction model is solely based on CT/CIT trials, it remains unclear whether it can be used to estimate relationships between MRD and PFS in trials using novel agents.

MRD assessment with novel agents

An overview of MRD data from clinical trials using novel agents can be found in Table 3.

Kinase inhibitors

Ibrutinib. The Bruton Tyrosine Kinase (BTK) inhibitor ibrutinib has shown promising results in previously untreated and relapsed/refractory (r/r) CLL patients and has therefore been widely approved as a monotherapy in both settings.^{32–35} While CR rates increase gradually during the course of treatment, they remain low and uMRD is only achieved by a minority of patients. Ahn and colleagues have reported a uMRD rate (FCM, <10⁻⁴) of 5/49 (10.2%; ITT-based: 5.8%) in PB after 4 years of ibrutinib treatment.³⁶ Additionally, the group compared PFS in patients with low MRD (<10⁻²) and high MRD (≥10⁻²) after 3 years of ibrutinib and no statistical difference could be shown.

While uMRD is not regularly achievable in ibrutinib monotherapy, combinations of ibrutinib with CIT or anti-CD20 monoclonal antibodies have shown higher uMRD rates. In the recent HELIOS trial of ibrutinib and BR (BR+I) vs BR only, the reported PB uMRD (FCM) rate at 36 months was 26.3% in the BR+I arm. The 3-year PFS rate for patients with uMRD was significantly higher than those for patients with MRD ≥ 10⁻⁴ (88.6% vs 60.1%).⁶

Other currently investigated front-line combinations include ibrutinib and rituximab (IR) and ibrutinib and obinutuzumab (IG). While uMRD seemed rare in IR, treatment with IG has shown a substantial PB MRD negativity rate of 35% in the recently published phase 3 iLLUMINATE trial.^{37–39}

Combinations of ibrutinib and anti-CD20-antibodies after an optional debulking with bendamustine were also tested in the CLL2-BIG and CLL2-BIO trial, in which the time-limited combination treatment of ibrutinib and obinutuzumab led to

Table 3**Overview of MRD Data from Relevant Studies of Novel Agents.**

Study	Treatment-naïve, relapsed/refractory, n	Treatment	MRD-guided treatment	MRD method/threshold	uMRD rate based on	
					MRD cohort ^a	ITT pop. ^b
Ahn <i>et al</i> 2018 ³⁶	TN (n=53), r/r (n=33)	Ibrutinib	no	Flow, 10 ⁻⁴ , PB	5/49 (10%)	5/86 (6%)
Cramer <i>et al</i> 2017 ⁴⁰	TN (n=40), r/r (n=25)	(Bendamustine), ibrutinib, ofatumumab	yes	Flow, 10 ⁻⁴ , PB	9/65 (14%)	9/66 (14%)
Jain <i>et al</i> 2017 ³⁷	TN (n=4), r/r (n=36)	Ibrutinib, rituximab	no	Flow, 10 ⁻⁴ , PB	NA	2/40 (5%)
Moreno <i>et al</i> 2018 ³⁸	TN (n=113)	Ibrutinib, obinutuzumab	no	Flow, 10 ⁻⁴ , PB/BM	NA	39/113 (35%)
von Tresckow <i>et al</i> 2018 ⁴¹	TN (n=30), r/r (n=31)	(Bendamustine), ibrutinib, obinutuzumab	yes	Flow, 10 ⁻⁴ , PB	29/61 (48%)	29/66 (44%)
Chanan-Khan <i>et al</i> 2016 ⁹¹	r/r (n=289)	Ibrutinib plus BR	no	Flow, 10 ⁻⁴ , PB/BM	76/211 (36%)	76/289 (26%)
Davids <i>et al</i> 2016 ⁹²	TN (n=35)	Ibrutinib plus FCR	no	Flow, 10 ⁻⁴ , BM	NA	29/35 (83%)
Jain <i>et al</i> 2018 ⁴²	TN (n=42)	Ibrutinib plus FCG	yes	Flow, 10 ⁻⁴ , BM	38/42 (90%)	38/43 (88%)
Wierda <i>et al</i> 2018 ⁴⁸	r/r (n=285)	Venetoclax	no	Flow, 10 ⁻⁴ , PB	46/174 (26%)	46/285 (16%)
Seymour <i>et al</i> 2018 ⁴⁹	r/r (n=194)	Venetoclax, rituximab	no	Flow/PCR, 10 ⁻⁴ , PB	121/167 (72%)	121/194 (62%)
Cramer <i>et al</i> 2018 ⁴⁴	TN (n=35), r/r (n=31)	(Bendamustine), venetoclax, obinutuzumab	yes	Flow, 10 ⁻⁴ , PB	55/63 (87%)	55/66 (83%)
Hillmen <i>et al</i> 2018 ⁵³	r/r (n=50)	Venetoclax, ibrutinib	yes	Flow, 10 ⁻⁴ , PB	23/40 (58%)	NA
Jain <i>et al</i> 2018 ⁵⁴	TN (n=80)	Venetoclax, ibrutinib	yes	Flow, 10 ⁻⁴ , BM	17/25 (68%)	NA
Rogers <i>et al</i> 2018 ⁹³	TN (n=25)	Venetoclax, obinutuzumab, ibrutinib	no	Flow, 10 ⁻⁴ , BM	18/23 (78%)	18/25 (72%)
Porter <i>et al</i> 2015 ⁵⁸	r/r (n=23)	Anti-CD19 CAR T-cells (CTL019)	no	HTS, 10 ⁻⁶ , PB/BM	4/14 (29%)	4/18 (22%)
Gauthier <i>et al</i> 2018 ⁵⁹	r/r (n=17)	Anti-CD19 CAR T-cells (JCAR014), ibrutinib	no	Flow, 10 ⁻⁴ , HTS, 10 ⁻⁶ , BM	Flow: 12/16 (75%); HTS: 10/16 (63%)	NA
Gill <i>et al</i> 2018 ⁶⁰	r/r (n=20)	Anti-CD19 CAR T-cells (CTL119), ibrutinib	no	HTS, 10 ⁻⁶ , BM	14/18 (78%)	14/20 (70%)
Siddiqi <i>et al</i> 2018 ⁹⁴	r/r (n=10)	Anti-CD19 CAR T-cells (liso-cel)	no	Flow, 10 ⁻⁴ , BM	6/7 (86%)	6/10 (60%)

r/r = relapsed/refractory, survival, TN = treatment-naïve, uMRD = undetectable minimal residual disease (<10⁻⁴).

^a Patients with available MRD testing.

^b According to iwCLL 2018².

PB uMRD in 48% (FCM, ITT-based: 44%) of the patients whereas the combination of ibrutinib and ofatumumab only yielded a PB uMRD rate of 14% (FCM, ITT-based: 14%).^{40,41}

An intensive treatment regime consisting of ibrutinib, fludarabine, cyclophosphamide and obinutuzumab (iFCG) has recently yielded a BM uMRD rate of 90% (FCM, ITT-based: 88%) after only 3 months of treatment in a cohort of IGHV mutated patients.⁴²

Undetectable MRD is rarely achieved in ibrutinib monotherapy, thus the assessment of MRD is not informative in this context. Its assessment is certainly reasonable in more intensive combination treatments using ibrutinib. As uMRD rates gradually improve over the course of ibrutinib treatment it may be sensible to set different time points for MRD assessment in trials using BTK inhibitors than those defined for CIT.

Other kinase inhibitors. No MRD data have yet been reported for the pivotal studies of the PI3K inhibitor idelalisib in combination with rituximab or with BR, as well as for the newer PI3K inhibitor duvelisib.⁴³ The currently ongoing CLL2-BCG trial will show whether deep remissions can be achieved by combining idelalisib with obinutuzumab. The CLLRumbrella1 and 2 studies will assess whether dual kinase inhibition (PI3K/ BTK in Umbrella 1 or SYK/BTK in Umbrella 2) in combination with obinutuzumab can lead to high rates of uMRD and thus MRD-based treatment termination.⁴⁴

Trials evaluating the use of other BTK inhibitors like acalabrutinib or zanubrutinib have not reported MRD data so far.⁴⁵

Venetoclax. The Bcl-2 inhibitor venetoclax has shown very impressive response rates in r/r CLL patients with or without high risk cytogenetics.^{46,47} A comprehensive MRD analysis of the two large phase II trials M13-982 and M14-032 evaluated venetoclax

monotherapy in mostly r/r CLL patients with or without del (17p).⁴⁸ MRD in PB was <10⁻⁴ (FCM) in 26% of the 174 patients with available MRD assessment. Among MRD-positive patients 23% showed intermediate ($\geq 10^{-4}$ to <10⁻²) MRD levels and 51% had high MRD ($\geq 10^{-2}$). PFS rates at 24 months were significantly higher for patients who achieved uMRD or intermediate MRD levels compared with patients who never achieved MRD < 10⁻² (92.8%, 84.3%, and 63.2% respectively).

Combinations of venetoclax with antibodies have yielded even higher rates of uMRD.⁴⁹ The MURANO study that investigated the use of rituximab and venetoclax in r/r CLL, showed uMRD in PB in 62% of all patients 2 to 3 months after the end of combination treatment with uMRD translating into considerably longer PFS.^{49,50}

In the CLL2-BAG study, an optional debulking with bendamustine was followed by an induction and MRD-guided maintenance treatment of venetoclax and obinutuzumab (VG) in patients with treatment-naïve or r/r CLL.⁴⁴ After induction treatment, a rate of PB uMRD of 87% (ITT-based: 83%) was achieved, with the majority of patients completing maintenance treatment at the earliest possible time point due to confirmed uMRD. The same drug combination has been investigated in the large phase III CLL14 trial. An interim safety analysis that comprised 12 patients showed PB uMRD in 10/12 (83%) patients.⁵¹ The phase II HOVON139 trial used the same combination with a different treatment scheme: after 2 months of obinutuzumab, 6 months of combined VG and 6 additional months of venetoclax 26 of 28 assessed patients (92.9%) achieved uMRD in PB.⁵²

The oral combination treatment of ibrutinib and venetoclax has also shown promising preliminary results. In the phase II CLARITY trial, 23/40 (58%) patients with r/r CLL have achieved PB uMRD (<10⁻⁴) after 12 months of combined therapy, out of which 13 (33%) have even reached MRD levels of <10⁻⁵.⁵³ In

another phase II trial of ibrutinib and venetoclax in treatment-naïve CLL patients, the reported rate of BM uMRD at 12 months was 17/25 (68%).⁵⁴ In both trials, the proportion of patients with uMRD increased steadily over the treatment course, promising even higher rates of uMRD with longer follow-up data available.

In summary combination treatments using ibrutinib and/or venetoclax yield high rates of uMRD in PB and BM that appear to increase with longer exposure to the targeted agents. First analyses have associated uMRD with increased PFS in patients treated with venetoclax-containing regimens, but more mature data from large trials are required to confirm the value of MRD status as an independent predictive factor for survival in the context of novel agents.

MRD assessment in allo-HCT and CAR T-cell therapy

Allo-HCT is a procedure with curative potential in CLL, yielding high rates of uMRD. Post-allo-HCT detection of residual disease is closely associated with impaired overall survival. In the prospective CLL3X trial, in which 90 patients have undergone allo-HCT, 10-year-OS was 51%, indicating that a certain proportion of patients can be considered cured after allo-HCT.⁵⁵ MRD status of these patients at the 12-month landmark post-allo-HCT highly correlated with long-term clinical outcome. Patients who had BM uMRD at this time point showed a significantly lower 10-year relapse incidence than those with detectable MRD (25% vs 80%, $p < 0.0001$).⁵⁶

Studies investigating anti-CD19 CAR T-cell therapies have yielded promising, but still limited results in extensively pretreated patients with CLL.⁵⁷ In the initial trial by Porter et al, 4 of 14 patients, who were treated with CAR T-cell therapy have achieved a CR with no evidence of MRD in PB and BM assessed by deep sequencing 1 or 3 months after CAR T-cell infusion. In none of the patients with uMRD and a CR relapse has been reported so far.⁵⁸

With the addition of ibrutinib to CAR T-cell therapy, high clinical response rates as well as a high proportion of patients with uMRD might be achievable. Gauthier et al report BM uMRD by flow cytometry in 12/16 (75%) of all evaluable patients who received additional ibrutinib vs 11/18 (65%) in patients not treated with ibrutinib.⁵⁹ In another recent study, 19 patients were treated with anti-CD19 CAR T-cells and ibrutinib. Of these, 15 patients had BM uMRD by flow cytometry and 14 by IGH sequencing 3 months after CAR T-cell infusion, at 12 months, 7 of 11 patients with evaluable BM remained without detectable MRD.⁶⁰

Limitations of MRD

MRD eradication: a goal for every patient?

In light of these data, absence of MRD is a goal for CLL patients, in particular when fixed-duration therapies are used. Given the excellent results obtained by continuous therapy with BTK-inhibitors such as ibrutinib, the question is whether MRD eradication should be a treatment goal in every patient.^{36,39,61}

Due to the median age of 72 years at diagnosis, the majority of CLL patients have comorbid conditions that may lead to ineligibility for intensive treatment regimens aiming at uMRD.^{1,62–65} In older patients with comorbidities and medication combinations of 2 or more antileukemic drugs may lead to higher toxicities and worse tolerability.⁶⁶ Thus, patients

with comorbidities, whose life expectancy is limited due to conditions other than CLL or simply due to advanced age might not always benefit from a maximization of treatment-free intervals but may profit from less intensive treatment strategies aiming at durable disease control rather than residual disease eradication at all costs. Despite low CR and uMRD rates, ibrutinib monotherapy has shown excellent progression-free survival (18-month PFS on ibrutinib in the pivotal RESONATE-2 trial: 89%, 2-year PFS in the ALLIANCE A041202 trial: 87%), durable remissions and a favourable safety profile as well as a considerable increase in patient-reported quality of life.^{39,67,68} These results demonstrate that achievement of uMRD may not be required to achieve long-term control of CLL.

Undetectable MRD is not equivalent to eradicated MRD

CLL is a disease that virtually always involves multiple compartments including blood, bone marrow, lymph nodes, spleen and liver, as well as cerebrospinal fluid, lung, bones, kidneys or skin.^{69–71} Several studies have shown that certain agents, in particular anti-CD20-antibodies, preferentially deplete cells in the peripheral blood and have less efficacy in other compartments.⁷² Consequently, MRD assessment in PB after anti-CD20-antibody-containing therapies might lead to an underestimation of residual disease across all compartments. In contrast, B-cell receptor pathway inhibitors (BCRi) like ibrutinib and idelalisib have been associated with a redistribution of tissue-residing CLL cells into the blood, often leading to a transient lymphocytosis.⁶¹ In light of these varying concordant results of residual disease assessment in bone marrow and peripheral blood, the EMA has stated that “if MRD is not detectable in PB, it is mandatory to confirm MRD status in the BM” in their approval of MRD status as an intermediate endpoint for licensure.¹⁴

However, even in absence of residual disease in the bone marrow, patients may have significant residual disease in unassessed sites. In a recent analysis, we have shown that residual abdominal lymphadenopathy after front-line CIT is associated with inferior PFS and OS regardless of MRD status, suggesting that in spite of uMRD, patients' lymph nodes act as a reservoir for residual disease and consequently cause relapse.⁷³

With current methods of MRD assessment showing limitations in detecting residual disease in the lymphatic system, the development of more sensitive methods like circulating tumour DNA (ctDNA)-based approaches is underway and promises a more comprehensive monitoring of disease burden across different topographical sites within the body.⁷⁴

Until these refined methods can be utilized it is sensible to continue using a multimodal approach that includes MRD assessment, physical examinations and imaging studies to estimate residual disease burden.

Predictive value of MRD status in relationship to disease biology

The predictive value of uMRD is influenced by several factors as treatment modalities, the residual disease site and particularly by subclones of the disease, which are not necessarily measured by a quantitative method like MRD assessment.^{75–79} Long-term follow-up data from the original phase II FCR cohort at MD Anderson Cancer Center (MDACC) showed a 12.8-year PFS rate of 79.8% for patients with mutated IGHV that had uMRD after

CIT.⁸⁰ Interestingly, patients with mutated IGHV, who were MRD positive had superior 12.8-year PFS compared to patients with uMRD and unmutated IGHV (36.9% vs 16.3%) despite the evident residual disease after completion of CIT.

These data are in line with the work of Dimier *et al* that established the validity of MRD status as a surrogate for PFS.⁵ Their calculations showed that approximately one third of the variability in the PFS hazard ratio (HR) could be attributed to the observed MRD results, which in turn implies that a considerable part of the variability is caused by factors other than MRD status, for instance disease biology.

These observations demonstrate that re-growth of the malignant clone and subsequent timing of relapse cannot be sufficiently described by the solely quantitative technique of MRD detection but needs to be complemented by biological information on the disease like IGHV, *TP53* status or other mutations like ATM, SF3B1, BIRC3, and NOTCH1.

Using MRD to guide treatment decisions

The improved treatment outcomes due to novel drugs allow further refinement and individualization of treatment strategies, possibly with the help of MRD assessment. Interim MRD analysis could be used to identify patients who benefit from treatment de-escalation or cessation to avoid unnecessary treatment-related toxicity. Similarly, assessment of MRD, together with genetic aspects, could identify patient groups in need of longer or more intensive treatment.

In BCR-ABL positive chronic myeloid leukemia (CML), MRD assessment is an integral part of the treatment strategy.⁸¹ Patients who achieve durable and deep molecular responses may discontinue treatment with BCR-ABL-directed TKIs with a good chance of entering a treatment-free period. Increase in BCR-ABL transcripts will prompt reinitiation of treatment in these patients. Although CLL does not have a single, targetable genetic aberration, a similar model could possibly be used in CLL in the future.

Currently evaluated MRD-guided treatment strategies

A retrospective analysis of the MDACC evaluated the outcome of FCR-treated patients who achieved uMRD after 3 (of 6 planned) treatment cycles.¹³ Patients with uMRD who stopped treatment after 3 cycles had similar PFS and OS as patients with uMRD who continued to receive additional FCR courses, suggesting that MRD-guided stopping of FCR treatment after 3 cycles is feasible without affecting long term survival and possibly sparing unnecessary treatment-related toxicities. Based on these observations and various sub-analyses, a risk-adapted treatment strategy for the first-line treatment of CLL is proposed, that includes interim MRD assessment as well as risk categorization on the basis of disease biology.⁷⁶ Besides an MRD-triggered end of treatment, the scheme envisages yearly follow-up MRD analyses and early treatment interventions in the case of molecular relapse.

While this exact strategy needs to be validated in the context of large prospective trials, further non-randomized MRD-guided approaches have already been tested. One trial evaluated the MRD-guided use of ibrutinib, fludarabine, cyclophosphamide and obinutuzumab (iFCG) in the first-line treatment of patients with a favourable genetic risk profile (IGHV mutated, no del(17p)/*TP53* mutation).⁴² After 3 courses of iFCG, BM MRD was assessed and patients with uMRD and CR (i) received 3 additional cycles of ibrutinib and obinutuzumab (IG) and 6

more cycles of ibrutinib (I). Patients who did not achieve uMRD, received 9 additional cycles of IG after iFCG, uMRD at 1 year prompted stopping of all therapy. Seventeen of 42 (40.4%) patients had uMRD and CR (i) and thus received the reduced intensity treatment while all 28 patients who reached the 12 months time-point had uMRD and stopped therapy per protocol.

In the CLL2-BAG trial, optional bendamustine debulking was followed by obinutuzumab (month 1–8) and venetoclax (month 2–8) induction treatment and a maintenance treatment consisting of the same agents with a flexible duration between 6 and 24 months.⁴⁴ Undetectable MRD in PB, confirmed by 2 subsequent FCM measurements in patients with (clinical) CR (i) led to treatment termination. At the end of induction treatment, 55/63 (87%) analyzed patients had achieved uMRD in PB and at the latest data cut, 21 patients had regularly finished maintenance treatment thanks to uMRD. The remissions seem durable after EOT, even in patients with genetic high risk features.⁸²

Another individualized approach to MRD-guided treatment decisions is evaluated by Hillmen *et al* in the context of the phase II CLARITY trial with ibrutinib and venetoclax (IV).⁵³ The group uses an innovative MRD-guided treatment scheme that extrapolates the optimal treatment duration from the time it took to achieve uMRD. Patients are tested for MRD after 6 and 12 months of combined treatment and continue IV for the same duration of time it took them to achieve uMRD. 24% of all patients showed uMRD in BM after 6 months and could thus finish treatment after 12 months of IV; PB uMRD rate at 12 months was 58%. This method of individualized treatment durations depending on time to uMRD is currently tested in the large phase III FLAIR trial comparing FCR, I, IR and IV in previously untreated CLL.⁸³

Another reasonable use of MRD could be the implementation of MRD-guided decision-making in the context of the now approved MURANO treatment scheme. It is not clear if MRD-positive patients should continue on single agent venetoclax while the data clearly show that patients with uMRD can stop after 24 months of venetoclax treatment.

MRD-guided treatment in CLL: too complex for routine practice?

As the example of CML treatment shows, an ideal MRD-guided treatment strategy in CLL would have to be simple, affordable, use widely available methods and improve treatment outcomes in terms of safety and/or efficacy.

The above mentioned examples of currently investigated MRD-guided treatment strategies seem to either improve tolerability by sparing drug exposure or efficacy by prompting extended treatment for those who require it. However, so far it is unclear how patients with detectable MRD after induction should be further treated. It has to be assessed whether these patients should just continue on the same treatment if applicable or if patients should change the substance, particularly when new subclones are detected.⁸⁴

Considering that in CLL not only MRD levels, but also genetic aberrations in subclones could be considered for future treatment approaches, it will be a challenge to transfer the complexity of these approaches into clinical practice.

Other than in BCR-ABL positive CML, CLL disease activity cannot be comprehensively captured by one method. Patients might for instance show fulminant nodal relapses that are not detectable by flow cytometry and the value of uMRD greatly depends on the therapeutic agents that are used. Furthermore, the

appropriate time points for MRD assessment have not yet been established in the context of novel agents and access to MRD detection methods is still limited.

Until a simple and cost-effective MRD-guided treatment approach is implemented in clinical routine, further exploration of MRD-guided treatment strategies in clinical trials will help to determine ideal time points to assess MRD and identify appropriate treatment durations for different patient groups. Once these groups are well defined, it might even be more practicable in routine patient care to use MRD-independent treatment schemes with different yet fixed durations that are selected depending on individual risk factors.

Outside of clinical trials there are only a few scenarios, in which MRD assessment is indicated or could be at least considered.

Highly sensitive assessment of residual disease is particularly vital in treatment settings aiming at cure of CLL like allo-HCT and CAR T-cell therapy. In allo-HCT continued serial assessments are useful to detect low-level molecular relapse that might influence immunosuppression or maintenance therapy.⁵⁵

MRD testing can also be useful in selected patients receiving highly effective combination treatments of novel agents, as venetoclax plus rituximab outside of clinical trials to evaluate treatment outcome and allow for prognostic estimations.^{44,50,52,53,82,85}

In contrast, in patients receiving indefinite treatments that rarely lead to uMRD (e.g. ibrutinib, idelalisib), MRD assessment might not be routinely indicated as it appears to lack predictive value.³⁶ Conversely, positive MRD results might worry patients and necessitate additional counselling.

According to the recently updated iwCLL guidelines, symptomatic disease remains the main criterion for treatment and MRD status should not play a role in establishing treatment indications outside of clinical trials and the post-allo-HCT setting.²

Whether MRD testing will indeed be utilized as comprehensively as currently foreseen, will ultimately depend on which treatment paradigm will prevail in the near future: time-limited combination therapies of novel agents aiming at uMRD or indefinite treatment with 1 substance aiming at durable disease control rather than eradication. Direct randomized comparisons of these 2 concepts are much-needed to advance this debate. Shanafelt et al recently suggested a survival benefit in patients treated with an indefinite ibrutinib-based regimen (IR) when compared to FCR-treated patients in the randomized E1912 trial, making a compelling case for indefinite treatment approaches even in fit patients.⁸⁶ Despite this data, indefinite treatment may come with drawbacks that are not captured by survival curves, an increased financial burden, higher cumulative toxicities or development of resistance mutations.^{76,87-89} In the light of the promising results from the above mentioned, time-limited and MRD-guided CLARITY (IV) and CLL2-BAG (GV) combinations, results from the phase III FLAIR (FCR vs I vs IR vs IV) and GAIA/CLL13 (FCR/BR vs RV vs GV vs GIV) trials are eagerly awaited to fuel this discussion with new data.^{83,90}

Conclusions

The addition of rituximab to chemotherapy and more recently the implementation of novel agents into first-line and relapse treatment have led to substantially prolonged survival in CLL patients, raising the need for new study and treatment endpoints. By allowing the use of MRD status as an intermediate endpoint for licensure, the European Medicines Agency has responded to

this need and established the basis for an expedited access to new CLL treatments.

Undetectable MRD and longer PFS also seem to correlate in first analyses of venetoclax-based regimens, while this association has not yet been shown for the first-line approved and widely used monotherapy with ibrutinib as well as for other kinase inhibitors. In any case, the predictive value of MRD status on survival has to be prospectively evaluated for all different treatment regimens, ideally including detailed analyses of different genetic subgroups.

New MRD-guided treatment approaches will probably soon find their way into clinical practice, allowing for a more individualized therapy, possibly leading to less treatment-related toxicities and better outcomes. Until this has become reality, it is necessary to eliminate the remaining weaknesses of current MRD assessment approaches and develop more sensitive methods to also detect residual disease beyond the BM and PB compartment.

The joint effort of ERIC and other CLL groups that has ultimately led to highly standardized methods for MRD detection by flow cytometry can serve as a model in this process to not only standardize the use of MRD assessment but to also make it more affordable and accessible in the routine clinical setting.

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